# EXHIBIT "B"

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| APPLICANT: | Pierre Belhumeur et al. |
| SERIAL NO.: | 09/980,649 |
| FILED: | June 4, 2002 |
| FOR: | BIOLOGICAL INDICATORS FOR VALIDATING A PRION STERILIZATION PROCESS |
| ART UNIT: | 1651 |
| EXAMINER: | Taeyoon Kim |

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DECLARATION UNDER 37 C.F.R. SEC. 1.132

I, Pierre Belhumeur, do hereby declare and state as follows:

1. I received the degrees of Bachelor (B.Sc.) of Microbiology from Laval University (Quebec, Canada) in 1981, Master (M.Sc.) of Microbiology and Immunology from University of Montreal (Montreal, Canada) in 1984, and Doctor of Philosophy (Ph.D.) of Molecular Biology from University of Montreal (Montreal, Canada) in 1989. I am presently Professor and Chairman of the Department of Microbiology and Immunology at the University of Montreal.
2. My academic background and experiences in the field of the present invention are listed on the enclosed *curriculum vitae*.
3. I am an author of several scholarly publications as listed in my enclosed *curriculum vitae*.
4. I have read the contents of U.S. Patent Application Serial No. 09/980,649 entitled "BIOLOGICAL INDICATORS FOR VALIDATING A PRION STERILIZATION PROCESS", including the claims presently pending.

5. Safar et al. studied the ***thermal stability*** and ***conformational transitions*** of scrapie amyloid protein and their ***correlation with infectivity***. For that, Safar et al. submitted scrapie amyloid protein to heat treatment and to chemical scrapie inactivators such as formic acid (FA), SDS, additional α-helix-inducing fluorinated alcohols and trifluoroacetic acid (TFA). Safar et al. demonstrated that the treatments that do affect the conformation of scrapie amyloid protein can also affect its infectivity. Their western blot analysis showed that some of these treatments produce dimer formation of scrapie protein.

6. U.S. Patent Application Serial No. 09/980,649 teaches ***a method for evaluating the efficiency of a sterilization process***, as claimed, and not measuring the conformational transitions of scrapie. Some sterilization methods produce a significant degradation of prion proteins whereas other sterilization methods produce a weaker degradation. The method claimed in the present patent application provides a means to evaluate the efficacy of different sterilization processes. The method presently claimed can be adapted to industrial processes where there is a need to control the efficiency of a sterilization process. By western blot analysis, the presently claimed method shows that there is no residual yeast prion protein detectable after ozone treatment (See, e.g. Figures 4 and 5 of U.S. Patent Application Serial No. 09/980,649), indicating degradation of same. In that respect, it differs from Safar et al. where no such observation of degradation has been made, or could be made, with their treatments.

7. Safar et al. showed that heat or chemical treatment can have an effect on the conformation of scrapie amyloid prion protein (PrP27-30, a mammalian prion protein) and that this level of change can be measured by Western blot analysis. Safar et al. is not

concerned with the evaluation of the efficiency of a sterilization process. In fact, the Western Blot analysis illustrated at figure 1 in Safar et al. illustrates the same quantity of prion protein as per the reported intensity of the various bands, providing no information as to possible degradation. In fact, Safar et al. only report figure 1 to illustrate the purity of the PrP27-30 protein (See, e.g. page 2207, left column, last paragraph).

8. The scrapie amyloid prion protein (PrP27-30) used by Safar et al. is a mammalian prion protein, whereas those used in U.S. Patent Application Serial No. 09/980,649 are yeast prion proteins. Substitution of one for the other may change any conclusion that can be made since there are significant differences between yeast prion proteins and mammalian prion proteins. There is a low level of homology between the amino acid sequences of mammalian prion proteins and yeast prion proteins. For example, the Sup35 yeast prion protein contains 685 amino acids (Kushnirov et al., Gene 66 (1), 45-54 (1988), submitted herewith as Exhibit A) while the human prion protein PrP is 253 amino acids long (Kretzschmar et al., DNA 5 (4), 315-324 (1986), submitted herewith as Exhibit B).
9. The yeast Sup35 protein binds GTP, is located in the cytosol and is a protein translation termination factor. On the other hand, the human prion protein PrP can bind copper, is a glycoprotein attached to the cell membrane and there are no indications of it being involved in protein translation.
10. The mechanisms of conversion of mammalian and yeast prions into their infectious forms differ significantly. The formation of the infectious form of the human prion protein PrP occurs after it transits into a subcellular compartment such as the lysosome while the change of conformation of the yeast prion proteins (e.g. Sup35) occurs in the cytoplasm. The mammalian prion protein changes conformation at pH 4.0 (the pH inside the

lysosome) while the conformational changes for the yeast prion protein occurs at physiological pH (c. pH 7.4).

11. While mammalian prion aggregates are essentially made of fibrils (resistant to detergents), there is no conclusive evidence that yeast prions are essentially made of fibrils.

12. The conformational changes of the yeast prion proteins require a molecular chaperone (protein Hsp104, for the Sup35 yeast prion protein) while there is no such requirement reported for the mammalian PrP prion protein. (Bousset et al., Microbes and Infection, 2002, 4: 461-469, submitted herewith as Exhibit C). These differences are significant.

13. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that wilful false statements and the like so made are punishable by a fine or imprisonment, or both (18 U.S.C. Sec. 1001), and may jeopardize the validity of the application of any patent issuing thereon.

Signed _____ Dated: September 25, 2009
Pierre Belhumeur